

# Analysis of a periplasmic thiol oxidoreductase in *Rhizobium leguminosarum*

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## Summary

In this work the expression and cellular localization of a predicted periplasmic thiol oxidoreductase encoded by *Rhizobium leguminosarum* 3841, ORF RL1083, were analysed. Based on the homology of the encoded protein with DsbA proteins from other bacteria we named it *dsbA*. The genetic organization of *dsbA* region showed that it is a monocistronic gene. A putative  $\sigma^{70}$  promoter was predicted upstream *dsbA* gene. The promoter region fused to *gusA* reporter gene revealed that *dsbA* is expressed in free-living conditions in different media and also, although at a lower level, in pea bacteroids. *R. leguminosarum* DsbA contains a potential Tat-dependent signal peptide. To localize this protein in different cellular fractions the protein was labelled by means of a C-terminal Strep tag. The DsbA-Strep protein was localized in the periplasmic fraction. At present three type of experiments are in progress: first, the study of DsbA Tat-dependence by using a *tat* mutant strain harbouring *dsbA*-Strep; second, the construction of a *dsbA* mutant and third the evaluation of periplasmic disulfide oxidoreductase activity of different strains: wild-type, *tat* mutant and *dsbA* mutant.

## Introduction

Periplasmic proteins play a very important role for the *Rhizobium*-legume symbiosis. Some of the proteins located in the periplasmic space require a translocation system designated Tat, able to translocate folded proteins containing signal peptides with a “twin-arginine” conserved motif (Sargent *et al.*, 1998). In *Rhizobium leguminosarum* 3841 more than one hundred proteins are predicted by bioinformatic tools to be transported by the Tat system (Jiménez, 2006). One of these is RL1083, a DsbA-like protein, that would catalyze disulfide bond formation in the periplasm. Dsb proteins are involved in plant and animal pathogenesis (Jiang *et al.*, 2008) and could have an important role in the *Rhizobium*-legume symbiosis.

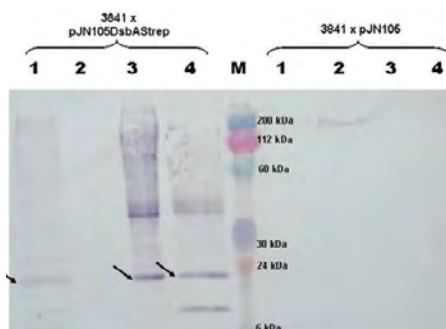
## Materials and Methods

Generation of a *gusA* fusion to *R. leguminosarum dsbA* promoter (plasmid pJP2pdsbA) was performed by cloning a 516-bp PCR DNA fragment containing 185 pb of *dsbA* promoter and 331 pb of *dsbA* gene in front of *gusA* gene of plasmid pJP2. A DsbA-Strep fusion protein was produced in *Rhizobium* using a derivative of plasmid pJN105 harbouring a DNA fragment encoding DsbA with a Strep-tag coding sequence fused to its C-terminus. DsbA-Strep was identified by immunoblot using Streptavidin conjugates. Disulfide oxidoreductase activity was determined by Ellman's assay (Jiang *et al.*, 2008). For more details on Material and Methods, see Vega (2009).

## Results and Discussion

Analysis of *R. leguminosarum dsbA* upstream region by NNPP (Neural Network Promoter Prediction server) predicted the presence of a putative  $\sigma^{70}$  promoter. Experimental analysis of this promoter using a fusion with *gusA* reporter gene indicated that *dsbA* is expressed in different media and in symbiosis with peas. These data are consistent with a constitutive expression of DsbA, suggesting that periplasmic protein disulfide bond formation will be required under most conditions. Additionally the promoter activities were determined at different OD<sub>600</sub> in free-living conditions (TY medium). The results indicated a similar activity at different OD<sub>600</sub> including exponential and stationary phases.

A protein compatible with the expected size of DsbA-Strep without signal peptide (26 kDa) was identified in total protein extracts and in periplasmic fractions by immunoblot. Also, this protein was partially purified from periplasmic cellular fractions by Streptactin chromatography (Figure).



**Figure.** Partial purification of DsbA-Strep protein by Streptactin chromatography. DsbA-Strep (arrows) was identified by Western blot using AP-conjugated Streptavidin. Lanes: **1**, Periplasmic proteins before chromatography. **2**, Streptactin column passthrough. **3-4**, Streptactin-binding proteins.

At present the Tat dependence of DsbA is being investigated using a *tat* defective strain (Meloni *et al.*, 2003). Generation of a *dsbA* mutant by recombination of a plasmid containing an internal fragment of *dsbA* gene is also in progress.

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### References

- Meloni S, Rey, L, Sidler, S, Imperial J, Ruiz-Argüeso T, Palacios JM (2003) *Mol Microbiol* 48: 1195-1207.
- Jiménez A (2006). Trabajo Fin de Carrera, Departamento de Biotecnología. Universidad Politécnica de Madrid.
- Jiang Jiang BL, Liu J, Chen LF, Ge YY, Hang XH, He YQ, Tang DJ, Lu GT, Tang JL (2008) *Mol Plant-Microbe Interact* 21: 1036-1044.
- Sargent F, Bogsch EG, Stanley NR, Wexler M, Robinson C, Berks, BC, Palmer T (1998) *EMBO J* 17: 3640-3650.
- Vega RM (2009) Trabajo Fin de Carrera. Departamento de Biotecnología. Universidad Politécnica de Madrid.